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Loss of coevolved basal and plastic responses to temperature may underlie trophic level host-parasitoid interactions under global change

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ABSTRACT

Climate change has complex impacts on insect life history traits, biogeography, survival, population dynamics and host-parasitoid seasonal phenologies that affect their synchrony. This is more pronounced in tropical southern Africa, where global warming may be relatively high. While some work on *Plutella xylostella* (L.) temperature tolerance has been reported, none focussed on African field insect populations and implications of trophic level interaction on biological control. To determine how climate change may affect coevolved trophic level interactions in a host-parasitoid trophic system, we compared the basal thermal tolerance of wild F_1 populations of the parasitoid *Cotesia vestalis* (Haliday) to two life stages of herbivorous host *P. xylostella* (L.), a global economic pest of brassicas. Our results showed significantly lower *C. vestalis* critical thermal limits ($P < .001$) compared to the host. Similarly, the parasitoid heat knockdown time and chill comma recovery time tolerance were significantly lower than that of the host ($P < .001$). Lethal temperature assays revealed significantly lower survival rates ($P < .001$) for *C. vestalis* at both upper and lower temperature scales. Observed differential basal temperature responses may offset coevolved host-parasitoid synchrony through differences in host-parasitoid phenologies. Hence, future biological control programmes should aim at 'creating' resilience and climate-fitness in natural enemies through enhancing evolutionary potential to buffer them from the changing climate. This is the first report detailing trophic level thermal tolerance of *P. xylostella* and larval parasitoid, *C. vestalis*, focussing on ecological service implications of the field-wild parasitoid populations on the herbivore host.

1. Introduction

The Earth's climate has significantly warmed in the past 100 years (Walther et al., 2002; IPPC, 2014) with predictions of 1.1–6 °C increase in atmospheric temperatures by the year 2100 (IPPC, 2014). Moreover, climate change has also brought increased temperature variability, with consequent complex often negative implications on ecological organisations (IPPC, 2014). Climate change has been reported to affect a broad range of insects at individual, community, population levels as well as bio-geographical patterns (Walther et al., 2002; De Meyer et al., 2008). Indeed insect physiology, population dynamics, behaviour and interactions with other species within the same environment is largely correlated with surrounding atmospheric temperature. This is so because all organisms are physiologically limited to defined temperature ranges within which their fitness is optimal (Ragland and Kingsolver, 2008).

Since fitness traits are expressed differentially in space according to both the G × E interaction and the Reaction Norm theories, thermal environmental variation shapes the evolution of fitness traits (Blanford et al., 2002; Vale et al., 2008; Wolinska and King, 2009). Species differences in thermal responses influence the intensity of co-evolution, thus creating co-evolutionary gaps in a host-parasitoid system and sometimes altering their co-evolutionary trajectories. This may shift their evolutionary optima and thus desynchronise the trophic balance (Thomas and Blanford, 2003; Vale et al., 2008). Hence, depending on their thermal sensitivity profiles, global climate change determines the stability and sustenance of trophic interactions in nature (Godfray, 1994; Hance et al., 2007; Bahar et al., 2012; Chidawanyika et al., 2012; Mutamiswa et al., 2017). These interactions evolved from, and rely on stable environmental conditions, especially temperature, to maintain their balance (Brodie et al., 2014).

Any changes in environmental temperature e.g. increased mean and variability coincident with climate change, may differentially alter the host or parasitoid thermal preferences and the synchronous phenologies, likely upsetting the delicate trophic balance and risking system imbalance (Godfray, 1994; Wolinska and King, 2009; Brodie et al.,

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2014; Mwalusepo et al., 2015). For a host-parasitoid system, this may upset ecological services and hence efficacy of biological control (Thomas and Blanford, 2003; Hance et al., 2007; Schreven et al., 2017). To stay in the 'contest' and maintain the phenological synchrony, the Red Queen hypothesis states that the host or the parasitoid has to keep adapting in the evolutionary landscape. However, the evolutionary landscape may not be static. This may mean that the environmental stress may disproportionally affect trophic system levels (Blanford et al., 2002; Wolinska and King, 2009).

Manifestations of trophic level host-parasitoid mismatches have been well elucidated (see Hance et al., 2007). Optimal phenological overlaps should remain within permissible thresholds to maintain ecological balance (Hance et al., 2007). This may be more pronounced in koinobiont and monophagous parasitoids that depend on one host (Girling et al., 2011) and require the host internal temperatures to remain within the permissible optimum range for survival (Brodie et al., 2014) like *Cotesia vestalis* (Haliday) (Hymenoptera: Braconidae). *Cotesia vestalis* is a cosmopolitan and solitary larval endoparasitoid of the diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) that has been widely reported in southern Africa (Kfir, 1997; Nofemela and Kfir, 2005; Nofemela and Kfir, 2006) and elsewhere (Shi et al., 2013), contributing 36–92% parasitism on *P. xylostella* (Kahuthia-Gathu et al., 2017). When the adult stage of the parasitoids are out of synchrony with the target life stage of the herbivore host; the parasitoid may face local extinction or may have reduced efficacy due to compromised environmental fitness, increasing risks of heightened host pest outbreaks (Hance et al., 2007).

Cotesia vestalis relative insecticide tolerance, abundance in African climates (Kfir, 1997; Nofemela and Kfir, 2006; Kahuthia-Gathu et al., 2017), high fecundity (Alizadeh et al., 2011), short life cycle coupled with high parasitism rates (Shi et al., 2013; Kahuthia-Gathu et al., 2017) and novel immunity inside the host (Gao et al., 2016) makes it potentially the main parasitoid agent for the global economic pest of brassicas, *P. xylostella* (Talekar and Shelton, 1993; Furlong et al., 2013) in warm tropical climates (Nofemela and Kfir, 2005; Nofemela and Kfir, 2006; Kahuthia-Gathu et al., 2017). Interestingly, *C. vestalis* is the most widely distributed *P. xylostella* larval parasitoids in Africa, Asia, Europe, America and Australia (Nofemela and Kfir, 2005; Nofemela and Kfir, 2006; Reddy, 2017).

Plutella xylostella is a global economic insect pest of brassicas (Talekar and Shelton, 1993; Furlong et al., 2013) with a seemingly increasing pest status in Southern Africa (Machekano et al., 2017). Increased pest status may be attributed to pesticide resistance (IRAC, 2015) and likely global warming (IPPC, 2014). While basal temperature tolerance of *P. xylostella* has already been reported (Shirai, 2000; Bahar et al., 2012; Nguyen et al., 2014; Marchioro et al., 2016; Ngowi et al., 2017), temperature effects on host-parasitoid interactions remain largely unknown (Hance et al., 2007). Studies with *P. xylostella* laboratory strains have shown that it has a broad temperature range of -16.5 to 42.6 °C (Nguyen et al., 2014). Moreover, models predict that projected climate change may likely enhance *P. xylostella* geographic range extension (Nguyen et al., 2014; Ngowi et al., 2017), against the backdrop of ~15–34% other insect species facing extinction (Thomas et al., 2004), with parasitoids being the most vulnerable (Hance et al., 2007; Thomas et al., 2004). However, among a myriad of biotic factors capable of regulating *P. xylostella* in nature (Nofemela and Kfir, 2006), parasitism constitutes about 48% contribution in population reduction (Nofemela and Kfir, 2005; Nofemela and Kfir, 2006; Marchioro and Foerster, 2011). As such, parasitoids remain the central tenet in *P. xylostella* management (Safraz et al., 2005; Gryzwacz et al., 2010; Furlong et al., 2013; Machekano et al., 2017). This is further supported by the continuous new reports on increased genetic insecticide resistance in *P. xylostella* (Zhao et al., 2006; Xia et al., 2014; Gao et al., 2016) which is exacerbated by the positive correlation between its abundance and insecticide use (Gryzwacz et al., 2010) and the prevailing favourable climate (Ngowi et al., 2017). Cognisant of these factors, it is imperative to enhance biological control using parasitoids, and enhance their climate resilience using evolutionary physiology (Chidawanyika et al., 2012; Sørensen et al., 2012).

Most of southern Africa lies in predominantly hot and dry bioclimatic region (Metzger et al., 2013). These bioclimatic conditions promote year round activity (Zalucki et al., 2012) specifically in the hot-dry spring (Nofemela and Kfir, 2005; Nofemela and Kfir, 2006) for *P. xylostella*, resulting in reduced generation time and increased pest activity (Marchioro and Foerster, 2011; Ngowi et al., 2017). It remains to be elucidated whether *P. xylostella* is physiologically constrained due to current climate change conditions in southern Africa (Marchioro and Foerster, 2011; Nguyen et al., 2014; Ngowi et al., 2017). In addition, little is known about the effects of these changing conditions on tightly interacting species e.g. *P. xylostella* and parasitoids. While studies of *P. xylostella* biological control are vast (Safraz et al., 2005; Gryzwacz et al., 2010; Furlong et al., 2013), and sufficient literature on the effectiveness of *C. vestralis* has been generated (Nofemela and Kfir, 2005; Kahuthia-Gathu et al., 2017; Reddy, 2017) none has investigated trophic level interactions to predict its biological control efficiency in a changing climate. Moreover, models predicting future *P. xylostella* distribution have also failed to account for the role of parasitoids (see Hance et al., 2007; Zalucki et al., 2012; Ngowi et al., 2017).

Studies on the evolutionary and ecological relationships between parasitoids and *P. xylostella* have been documented (see e.g. Thomas and Blanford, 2003; Bahar et al., 2013; Schreven et al., 2017). However, the effect of temperature on these relationships has been neglected since they are not directly observable. It remains unclear whether or not field populations of *C. vestalis* have co-evolved similar thermal tolerance capacities and thermal plasticity as their *P. xylostella* host under changing climates. While similar studies have been done for other herbivorous host-parasitoid models, results have been largely equivocal and thus the interpretations drawn cannot be generalised (Hance et al., 2007; Brodie et al., 2014), hence warranting more individual studies (Karban, 1998; Hance et al., 2007). Furthermore, most studies to date, have focused on laboratory populations, whose fitness traits may be modified through laboratory adaptation (Hoffman and Fisher, 1994), hence compromising conclusions drawn from such studies. This warrants more robust studies, using ecologically relevant wild populations to better understand trophic level interactions in the face of global change. In the current study, we used F_1 wild populations to compare the basal thermal tolerance limits between host *P. xylostella* and parasitoid *C. vestalis* to establish whether the host or the parasitoid has higher thermal tolerance than the other and whether these tolerance capacities are liable to the same changes under global warming; which would indicate survival chances of either insect species. To our knowledge, this is the first report detailing trophic level interactions between the two, using F_1 ecologically-relevant study animals. Such information is critical to improve our ability to predict host-parasitoid interactions and efficacy of biological control thereof in the face of climate change. We hypothesize that the parasitoid's thermal tolerance matches that of the herbivorous host because they have coevolved under the same environment.

2. Materials and methods

2.1. Insect cultures

Pupal cocoons of *P. xylostella* and *C. vestalis* were collected from three high brassica production farms in Botswana: Noka Farm, Central District (S21, 12860; E027, 48830); Ambrose Phuthi Farm, Kgatleng District (S24, 54720; E026, 00909), and NAMPAAD Horticultural Demonstration Centre, Central District (S22, 48075; E027, 22591). The cocoons were collected at around the same time in 2015 austral summer. These sites are thermally homogeneous with a general range of 3.4–35.5 °C, mean minimum monthly temperature range of 11.9–13.3 °C, mean maximum monthly temperature range of

29.1–30.4 °C and mean monthly temperature range of 20.5 °C–22.6 °C. Using camel hair brushes and tweezers, balanced number of pupae for each species were collected from each of the farms. The petri dishes containing pupae were placed inside 32 cm^3 (*P. xylostella*) and 25 cm^3 (*C. vestalis*) insect cages (BugDorm®, MegaView Science Co., Ltd. Taiwan) and allowed to eclose at 27 ± 1 °C and 65 ± 5% RH in Memmert climate chambers (HPP 260, Memmert GmbH + Co.KG, Germany). Emerged *C. vestalis* adults were fed on 10% honey solution while *P. xylostella* adults were fed on 10% sucrose solution *ad libtum* using the dental wick source method. *Plutella xylostella* adults were supplied with four weeks organically produced cabbage *Brassica oleracia var capitata* seedlings, Star 3301 variety (Starke Ayres) to lay eggs in 60 cm^3 rearing cages at 27 °C and 65 ± 5%. Hatched larvae were allowed to grow on a constant supply of these organically produced cabbage seedlings as food source. About 100 4th instar larvae were transferred to cages with *C. vestalis* larvae for parasitism every week. Experiments were conducted using F_1 generation 4th instar larvae, ± 5 day-old adults for *P. xylostella* and ± 2 day-old adults for *C. vestalis* (de Boer et al., 2007). For experimental purposes, sex was not considered a factor in both species since it has been reported not to affect thermal tolerance traits in *P. xylostella* (Chang et al., 2012) and other species (e.g. Nyamukondiwa and Terblanche, 2009; Bertoli et al., 2010). Most previous studies on the effect of temperature and the interaction of *P. xylostella* with its natural enemies did not also consider sex (Dosdall et al., 2012; Bahar et al., 2012; Schreven et al., 2017) including those focussing on *P. xylostella* thermal tolerance alone (Shirai, 2000; Nguyen et al., 2014; Zhang et al. (2015a,b)) or *C. vestalis* alone (Htwe et al., 2008; Shi et al., 2013).

2.2. Critical thermal limits (CTLs)

CTLs were assayed using a programmable waterbath (LAUDA Ecogold® RE 2025, Lauda-Königshofen, German) as outlined by Nyamukondiwa and Terblanche (2009) connected to a transparent double-jacketed chamber. A thermocouple (type K 36SWG) connected to a digital thermometer (Fluke 54 series IIB) was inserted into the central organ pipe (control chamber) to record chamber temperature. A total of ten test insects replicated three times to yield 30 replications per treatment were used in these experiments. Test insects were individually placed into the organ pipes on the double jacketed chamber connected to a programmable waterbath filled with 1:1 water: propylene glycol to allow for sub-zero temperatures (Chown and Nicolson, 2004). Both critical thermal maxima (CT_{max}) and critical thermal minima (CT_{min}) experiments were started from an ambient set point temperature of 27 °C from which temperature was ramped up (CT_{max}) or down (CT_{min}) at 0.12 °C/min, 0.25 °C/min and 0.50 °C/min until CTLs were recorded (Terblanche et al., 2007; Terblanche et al., 2011; Allen et al., 2016). The start point of 27 °C was informed by the field insect source sites' annual mean temperatures. In this study, we defined the CTLs as the temperature at which each individual insect lost coordinated muscle function and the ability to respond to mild stimuli (e.g. prodding with a thermally inert object).

2.3. Heat knockdown time (HKDT)

The HKDT experiment was conducted following established methods by Weldon et al. (2011) and validated by Overgaard et al., (2012). Ten insects were placed in labelled 2 mL eppendorf tubes and exposed to 50 ± 0.5 °C and 65 ± 5% RH (12:12) (L:D) in Memmert® HPP 260 constant climate chambers. This heat knockdown test temperature was informed by preliminary experiments on CT_{max} and was observed sufficient to elicit heat shock. The climate chamber was equipped with a 1.3 MP WDR Pinhole Covert Network HD camera (DS-2CD6412FWD-20, Hikvision Digital Technology Co., Ltd, China) connected to a computer and timed by a stopwatch (Hinckley and Schmitt® sports timer) from which observations were made. The experiment was immediately repeated three times for each life study animal to give a total of 30 replications. HKDT was defined as the time (in minutes) at which insects lost coordinated muscle activity following heat stress.

2.4. Chill coma recovery time (CCRT)

CCRT was assayed following a method developed by Weldon et al. (2011) with necessary modifications. Ten individual insects (replicated three times) were placed in 2 mL eppendorf tubes and placed into a 33 x 22 cm zip-lock bag which was plunged into a Merck® water bath (Modderfontein, South Africa) set at -5.0 °C for 1 h. This chill coma temperature was informed by preliminary experiments based on low temperature activity. Temperature inside the bath was monitored by placing a thermocouple (type K 36 SWG) connected to a digital thermometer (Fluke 54 series IIB) into a control 11th eppendorf tube. After 1 h, the tubes were then placed in a Memmert® climate chamber set at benign 27 ± 1 °C, 65 ± 5% RH for recovery and connected to a camera. CCRT was defined as the time (in minutes) taken for test insects to stand rightly on their legs (Bertoli et al., 2010; Weldon et al., 2011).

2.5. Super cooling points (SCPs)

SCPs were measured following established methods by Nyamukondiwa et al. (2013). Sixteen individuals for each species were individually loaded into 1.0 mL microcentrifuge tubes. Inside the eppendorf tube, each insect was placed in contact with the tip of a type-T copper-constantan thermocouple (36-AWG; Omega, Laval, Canada), inserted through the tube's lid and both the insect and the tip of the thermocouple were held in place with cotton wool. Thermocouples were then connected to one of two 8-channel Picotech TC-08 (Pico Technology, Cambridge, UK) thermocouple interfaces and temperature recorded at 1 Hz using PicoLog software for windows. Each tube was then placed into a ziplog bag and then into a programmable waterbath (LAUDA Ecogold® RE 2025, Lauda-Königshofen, German) containing 99.9% ethanol. The waterbath was ramped down at 0.25 °C/min from 15 °C until SCPs for each test organism was recorded. SCPs for each individual were determined as the lowest temperature recorded prior to a spike in temperature associated with the latent heat of crystallization (see Nyamukondiwa et al., 2013).

2.6. Lethal temperature assays and microclimate data records

Lethal temperatures were determined using established methods as outlined in Chidawanyika and Terblanche (2010). Upper and lower lethal temperatures (ULTs and LLTs) were determined using direct plunge protocol on a range of durations (0.5 h, 1 h, 2 h and 4 h) and temperatures that gave 0–100% mortality across all tested temperature-time interactions. In light of reports of possible increasing temperatures (Steynor et al. 2012; Metzger et al., 2013; IPPC, 2014) due to climate change especially in Southern Africa (Zalucki et al., 2012; Metzger et al., 2013; IPPC, 2014) our primary focus on lethal temperature assays was on high temperature (ULTs) only. However, for the sake of completeness, we also tested a range of low temperatures (LLTs) for all the test insects for a 2-h duration. Ten insects were placed in 60 mL polypropylene vials with gauzed lids and placed in a 33 x 22 cm zip-lock bag, replicated three times. This was then plunged into a Merck® water bath (Modderfontein, South Africa) with 99.9% Ethanol. For ULTs, tiny wet filter paper was suspended in each vial to maintain benign humidity and prevent desiccation-related mortality. Following treatment (ULT and LLT), test insects were placed in a 27 ± 1 °C and 65 ± 5% RH in a Memmert® climate chambers for 24 h before scoring survival. All insects had access to food and water *ad libtum* during the 24 -h recovery period. Survival was defined as ability to pupate (for larvae) and coordinated muscle response to stimuli such as gentle prodding, or normal behaviours such as feeding, flying or mating for adults (Chidawanyika and Terblanche, 2010; Nyamukondiwa et al., 2013).

Table 1
Effects of treatment (*C. vestalis* adult and *P. xylostella* larvae and adults) on critical thermal limits (CT_{min} and CT_{max}). Tests of significance were done using factorial ANOVA and statistically significant means were separated using Tukey's HSD test at 95% CI. DF = Degrees of freedom, SS = Sum of Squares, MS = Mean Sum of Squares, F = Fisher–Snedecor test statistic.

Parameter	DF	SS	MS	F	*P*-value
Critical Thermal Maxima					
Treatment	2	528.0	264.0	99.3	< .001
Ramping rate	2	692.1	346.0	130.2	< .001
Treatment × Ramping Rate	4	360.9	90.2	33.9	< .001
Critical Thermal Minima					
Treatment	2	1227.0	613.6	588.7	< .001
Ramping rate	2	64.0	31.9	30.6	< 0.001
Treatment × Ramping Rate	4	6.0	1.5	1.5	.208

Shaded microclimate temperature data were recorded from Ambrose Phuthi Farm (see Section 2.1) using thermocron i-buttons (Fairbridge Technologies, UK). Temperature data was recorded consistently every 1 h for ~ 12 months.

2.7. Statistical analyses

Data were analysed in STATISTICA 13.0 (Statsoft Inc., Tulsa, Oklahoma) and R version 3.3.0 (R Development Core Team, 2016). Upper and lower lethal temperature limits (ULT and LLT), SCPs, HKDT and CCRT data were analysed using generalised linear models (GLM) assuming a binomial (for lethal temperature assays) and Gaussian (SCPs, HKDT and CCRT) distribution and a logit link function in R3.3.0 statistical software (as in Chidawanyika and Terblanche, 2010). CTLs met the linear model assumptions of constant variance and normal errors, so the data were analysed using factorial ANOVA in STATISTICA 13.0. Tukey-Kramer's *post hoc* test was used to separate statistically heterogeneous means. Temperature data graphs and analysis were done in OriginPro 8 and MATLAB 7.1.2 softwares.

3. Results

3.1. Critical thermal limits (CTLs)

3.1.1. Critical thermal Maxima (CT_{max})

The treatments (*C. vestalis*, *P. xylostella* larvae and adult) and ramping rate had highly significant effects ($P < 0.001$) on CT_{max} (Table 1). *Cotesia vestalis* had generally lower CT_{max} relative to *P. xylostella* larvae and adults (Fig. 1A). Slower ramping rates significantly compromised CT_{max}, and the slowest ramping rate (0.12 °C/minute) had most pronounced negative CT_{max} effects on *C. vestalis* relative to host *P. xylostella* (Fig. 1A). At ramping rate of 0.25 °C/min, the CT_{max} for *C. vestalis* was 44.29 ± 0.61 °C which was significantly lower ($P < 0.001$) than both *P. xylostella* larvae, 45.57 ± 0.51 °C and adult 46.55 ± 0.52 °C. The interaction between treatment and ramping rate was highly significant ($P < 001$) for CT_{max} (Table 1: Fig. 1A).

3.1.2. Critical thermal minima (CT_{min})

Treatment and ramping rate were all highly significant for CT_{min}. However, the treatment × ramping rate interaction effect was not significant ($P = 0.208$) (Table 1). Slower ramping rates (0.12 °C/minute) significantly improved CT_{min} across all the treatments tested here. Nevertheless, there was no significant difference for CT_{min} between the two fastest ramping rates, across all tested treatments (Fig. 1B). *Cotesia vestalis* had significantly higher CT_{min} (compromised low temperature survival) (1.73 ± 0.40 °C) as compared to the host larvae (−2.36 ± 0.53 °C) and adult (−3.23 ± 0.41 °C). Generally, the parasitoid showed a narrow activity temperature range of 1.73 ± 0.40–44.29 ± 0.61 °C compared to −2.36 ± 0.53–45.57 ± 0.51 °C (host larvae) and −3.23 ± 0.41–46.55 ± 0.52 °C (host adult) signifying an activity temperature difference of 5.35 °C and 7.22 °C with host (*P. xylostella)* larvae and adult respectively (Fig. 1A and B).

3.2. Heat knockdown time (HKDT), chill comma recovery time (CCRT) and supercooling points (SCPs)

Treatment had significant effects on high temperature tolerance, measured as HKDT ($\chi^2 = 197.11$, df = 2, $p < .001$). *Plutella xylostella* larvae had significantly higher HKDT relative to other 2 treatments, *C vestalis* and *P. xylostella* adults (Fig. 2A). Nevertheless, there was no significant difference between *C. vestalis* and *P. xylostella* adult HKDT (Fig. 2A). The time (minutes) needed to recover mobility (at 27 ± 1.0 °C and 65 ± 5.0% RH) from cold shock (chill coma) (−5 °C for 1 h) between the parasitoid and the host was significantly different ($\chi^2 = 16.344$ df = 2, $p < .001$). *Cotesia vestalis* took significantly longer time (3.40 ± 0.381 mins) to recover from chill comma compared to both *P. xylostella* larvae (2.91 ± 0.42 mins) and adult (2.48 ± 0.40 mins) (Fig. 2B) indicating higher vulnerability of the parasitoid to effects of cold stupor relative to the host. However, the host, *P. xylostella* larvae and adults were not significant.

There was also a significant treatment effect for SCPs (Fig. 2C). The SCP for adult *P. xylostella* (−18.66 ± 0.48 °C) was significantly lower

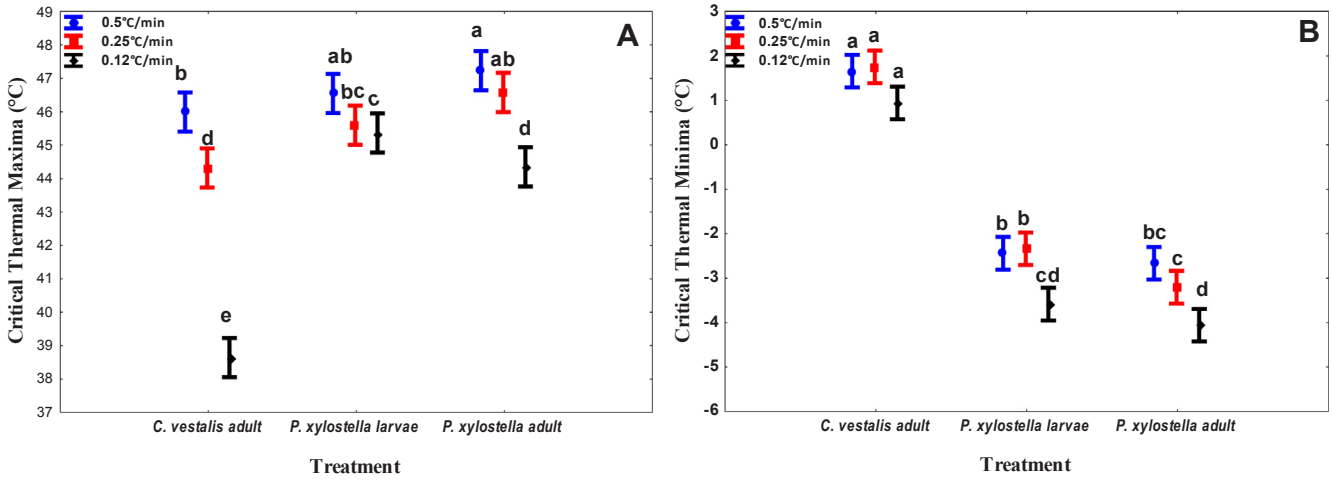

Fig. 1. Effects of ramping rate (0.12, 0.25 and 0.5 °C/min) and treatment (*Cotesia vestalis* adults, *Plutella xylostella* larvae and adults) on Critical Thermal Limits (CTLs) (mean ± SE); (A) Critical Thermal Maxima and (B) Critical Thermal Minima. Means with the same letter(s) are not statistically significant (Tukey-Kramer's HSD test at 95% CI).

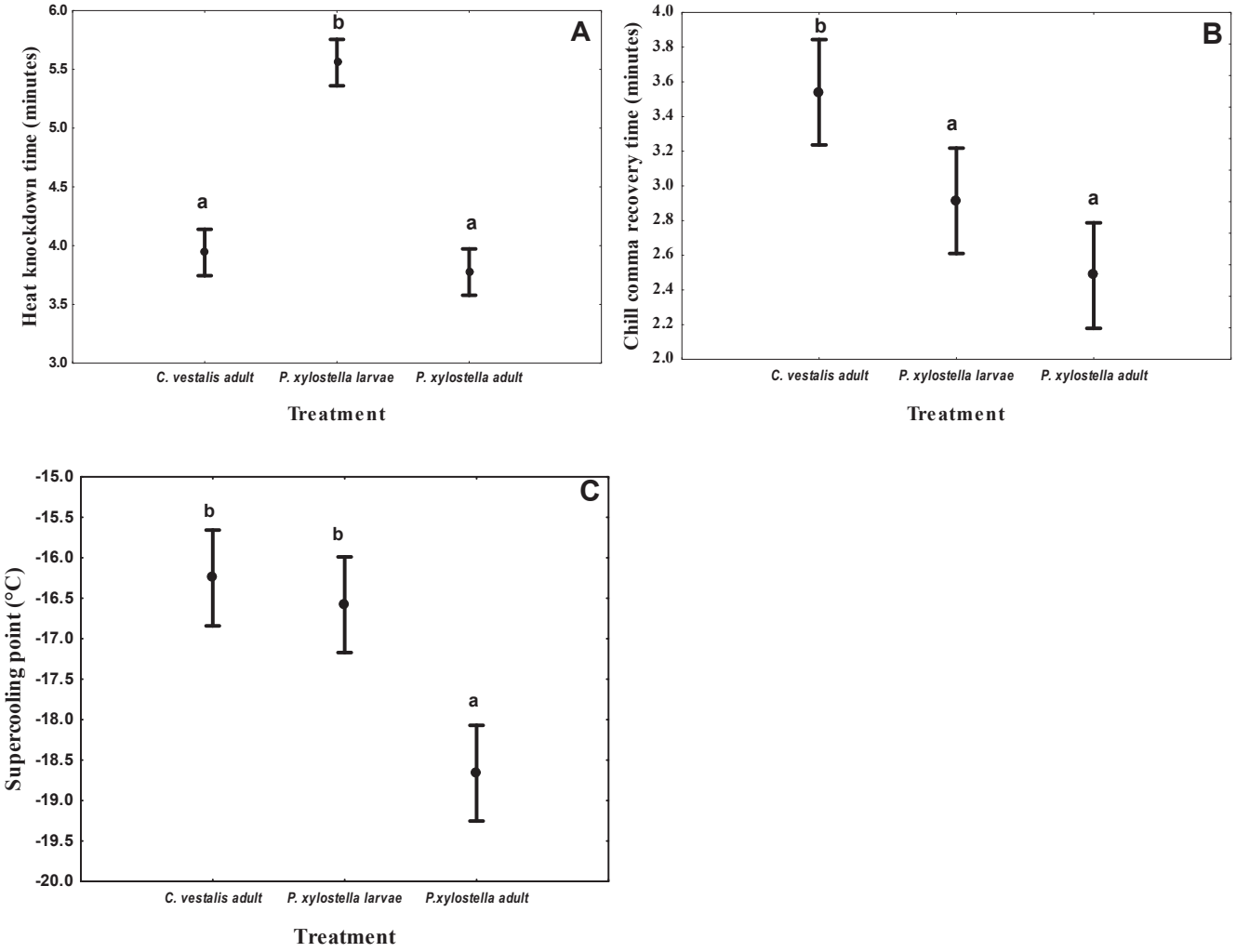

Fig. 2. Effect of treatment (*Cotesia vestalis* adults, *Plutella xylostella* larvae and adults) (mean ± SE) on (A) Heat Knockdown time (B) Chill Comma Recovery Time, and (C) Supercooling Points. Means with the same letters are not statistically significant (95% CI).

(χ^2 = 38.388, df = 2, p < .001) than both its larvae (−16.58 ± 0.48 °C) and the parasitoid (−16.25 ± 0.64 °C). However, no significant differences were observed between the SCPs of *C. vestalis* and the host larvae (Fig. 1C).

3.3. Lethal temperature assays

3.3.1. Upper lethal temperatures (ULTs)

There was a general decrease in survival as temperature and duration of exposure increased for all treatments (*Cotesia vestalis* adults, *Plutella xylostella* larvae and adults) (Table 2). However, treatments x time interaction effects were not significant for all test durations for ULT assays (Table 2). For all durations *C. vestalis* survival was lower with a narrower survival temperature range compared to all life stages of *P. xylostella*. At 0.5 h, *C. vestalis* survival ranged from ULT_{100} (39 °C) to ULT_0 (45 °C) compared to *P. xylostella* larvae ULT_{100} (41 °C) to ULT_0 (47 °C) and adult ULT_{100} (43 °C) to ULT_0 (47 °C) (Fig. 3A). Within each species, significant differences in survival between successive test temperatures were observed from 41 to 45 °C (Table 2; Fig. 3A). At 1 h, *C. vestalis* survival ranged from 35 °C (ULT_{100}) to 43 °C (ULT_0) compared to *P. xylostella* larvae ULT_{100} (40 °C) to ULT_0 (45 °C) and adult ULT_{100} (40 °C) to (ULT_0) (43 °C) (Fig. 3B). A similar trend was also observed at 2 h, where *C. vestalis* survival ULT_{100} was at 34 °C and ULT_0 was recorded at 43 °C as compared to *P. xylostella* larvae ULT_{100} (38 °C) and ULT_0 (43 °C) and adult ULT_{100} (37 °C) to ULT_0 (43 °C) (Fig. 3C).

When duration of exposure was doubled to 4 h, there was a wider margin in survival (Fig. 3D) between *C. vestalis* and both life stages of *P. xylostella*. *Cotesia vestalis* ULT_{100} was at 31 °C to ULT_0 (42 °C) as compared to *P. xylostella* larvae ULT_{100} (36 °C) to ULT_0 (43 °C) and adult ULT_{100} (37 °C) to ULT_0 (43 °C) (Fig. 3D). Significant differences within species (Table 2) in ULT was also observed for *P. xylostella* (Fig. 3A; C; D). However, the *P. xylostella* larvae generally exhibited higher survival than the adult (Fig. 3C; D) (Table 2). Generally the parasitoid, *C. vestalis* succumbed to high temperature faster than the host *P. xylostella* larvae and adults and differential high temperature tolerance effects were more pronounced for the 1 h and 4 h durations (Fig. 3A–D).

3.3.2. Lower lethal temperatures (LLTs)

Treatment and temperature had significant effects on low temperature tolerance, measured as LLT (Table 2; Fig. 3E). Parasitoid, *C. vestalis* had significantly lower (χ^2 = 101.92, df = 2, p < .001) survival than both the larvae and the adult host *P. xylostella* (Table 2; Fig. 3E) at low temperature stress. *Cotesia vestalis* LLT_{100} = 2 °C and LLT_0 = −17 °C, and LLT_0 was 2 °C and −17 °C for both *P. xylostella* larvae and adult.

3.4. Field microclimate temperature data

Annual microclimate data showed temperature ranges between −5.95 °C and 49.58 °C (Fig. 4A). Minimum temperatures of −5.95 °C

Table 2
Effects of treatment (*Cotesia vestalis*, *Plutella xylostella* larvae and adults), temperature and their interaction effects on upper lethal temperature (ULT) and lower lethal temperature (LLT) at different durations of exposure. Tests of significance were done using ANOVA under generalised linear models (GLM) analysis assuming logit link function in R. Three replicates of ten individuals were used (n = 30; N = 1800 test insects). DF = degrees of freedom.

Trait	Duration (h)	Effect	DF	Wald's χ^2	*P-value*
ULT	0.5	Treatment	2	109.62	< .001
		Temperature	14	1050.04	< .001
		Treatment × Temperature	28	24.25	.6681
	1	Treatment	2	283.09	< .001
		Temperature	14	1282.28	< .001
		Treatment × Temperature	28	16.91	.9504
	2	Treatment	2	50.20	< .001
		Temperature	14	1269.70	< .001
		Treatment × Temperature	27	-19,439.00	1
	4	Treatment	2	334.87	< .001
		Temperature	15	1287.80	< .001
		Treatment × Temperature	30	19.89	.9193
LLT	2	Treatment	2	101.92	< .001
		Temperature	16	1128.88	< .001
		Treatment × Temperature	32	48.57	.0346

were recorded in August, and highest minimum was (17.13 °C) (in December) with considerable monthly fluctuations, while the mean monthly temperatures ranged from 12.37 °C in July to 28.23 °C in December. Similarly, maximum temperatures ranged 30.13 °C in July to 49.58 °C in November-January (Fig. 4B). Using MATLAB 7.1.2 software, we determined the number of times the temperatures were $\geq CT_{max}$ and $\leq CT_{min}$ for each of the test insects. Observed microclimate data showed temperatures often crossed *C. vestalis* CT_{max} 281 times in summer, relative to *P. xylostella* larvae and adult (217 and 171 times respectively) for the same period (Fig. 4A; B). Similarly, temperatures were 198 times $\leq CT_{min}$ for *C. vestalis,* and 31 and 18 times $\leq CT_{min}$ for *P xylostella* larvae and adult respectively during winter season.

4. Discussion

The study showed that field populations of *C. vestalis* had narrower basal thermal tolerance compared to the herbivorous host, *P. xylostella*. The parasitoid's lower CT_{max} and higher CT_{min}, compared to the host suggest that it is prone to lethal temperatures and loss of motor control earlier than the herbivorous host. Loss of motor activity earlier in time makes the parasitoid more vulnerable to predation, starvation, reduced mating and risk of high mortalities that are likely to reduce its survival relative to the host. We recorded shorter HKDT and longer CCRT for the parasitoids relative to its host, indicating the parasitoid succumbed more to heat shock relatively quicker while taking longer to recover from cold shock, with likely negative consequences on its fitness, efficacy and ecological services in general. Similar observations were recorded for ULTs and LLTs, indicating a survival advantage for the host pest relative to its antagonist under both extremes of temperature (see Hance et al., 2007; Dosdall et al., 2012; Schreven et al., 2017). Microclimatic data records show that field temperatures surpass thermal functional limits for the parasitoid more often than that of the host, indicating a higher thermal stress for the parasitoid relative to the host. To our knowledge, this is the first report, detailing trophic level thermal tolerance of herbivorous *P. xylostella* and its tropical larval parasitoid, *C. vestalis*, exploring ecological service implications of the parasitoid. The present study presents novel ecologically relevant results closely approximating natural host-parasitoid interactions in the agro-ecosystem.

We recorded a significantly enhanced temperature tolerance capacity for host *P. xylostella* relative to the parasitoid *C. vestalis* measured as CTLs (CT_{max} and CT_{min}). Our results are corroborated by Chang et al. (2012) who indicated laboratory strain of *P. xylostella* CT_{max} to be very high; 50.31 °C; 45.76 °C and 47.73 °C for 4th instar larvae, adult females and males respectively without significant differences between the sexes. Nevertheless, to our knowledge, there is currently no data on *C. vestals* critical limits to activity. Life-table studies have revealed higher (between 8.14 and 11 °C) (Htwe et al., 2008) and lower (33–38.2 °C) (Htwe et al., 2008; Marchioro et al., 2016) minimum and maximum developmental thresholds respectively for *C. vestalis* relative to *P. xylostella* (minimum 6.1–8.0 °C and maximum of up to 45–50.31 °C) (Chang et al., 2012; Bahar et al., 2013; Nguyen et al., 2014), in agreement to our findings here. In a similar study, Bahar et al. (2012) also reported higher increase in *Diadegma insulare* mortality with increasing temperature than *P. xylostella*. High temperature tolerance by *P. xylostella* could be a result of its genetic superiority (Lee, 2013). *Plutella xylostella* has a big genome size with extra 1412 genes that are specifically unique to it that have extreme ability to quickly process environmental information and alter biological pathways (Lee, 2013). Furthermore, Sonoda and Tsumuki (2008) reported that *P. xylostella* has a unique family of genes that elicit significantly increased expression of *Hsp*70 at extreme temperatures (Zhang et al. (2015a,b)) or in response to parasitism (Bahar et al., 2013). The reason for this is unknown. Nevertheless, potential for monophagous *C. vestalis* survival within host above CTLs and the role of *Hsps* still warrants further investigation.

Fluctuating temperatures provide a relatively more accurate assessment of insect developmental biology than constant ones (Dosdall et al., 2012), and in nature, temperatures often vary by different magnitudes (Brodie et al., 2014). Slower ramping rates significantly compromised CT_{max} in both *C. vestalis* and *P. xylostella*, indicating lack of short-term plasticity, otherwise termed rapid heat hardening (Kelty and Lee, 2001). The magnitude of cost of slower ramping rate on CT_{max} was more pronounced in the parasitoids than host (Fig. 1A), consistent with reports by Gercken et al. (2015). Reduction of CT_{max} at slow ramping rate is still debatable in literature (Terblanche et al., 2007) but it may be a result of higher costs (longer time of exposure to heat stress translating to extended time of engaging stress response mechanisms and thus 'unaffordable' larger energy costs at low ramping rates) (Sørensen et al., 2013). For example, *C. vestalis* begins to upregulate its Hsp70 at 27 °C (Shi et al., 2013); the energy budget may not suffice to offset the cost at low ramping rates from such a 'low' temperature to a CT_{max} of 44.29 °C. In principle, insects would, therefore, forego the cost at low ramping rates and thus succumb earlier to stress hence lower CT_{max}. Bahar et al. (2012) also recorded high *Hsp*70 expression and high heat stress tolerance in both *P. xylostella* and *D. insulare* abruptly exposed to high temperature than those subjected to ramping temperatures. This may possibly explain why higher ramping rates (synonymous to 'heat shock') resulted in higher CT_{max} in our study. Similarly, slow ramping rate were associated with higher accumulation of heat damage on cells (Sørensen et al., 2013), whose magnitude is a function of duration of exposure to heat stress.

Conversely, slower ramping rates improved low temperature tolerance, measured as CT_{min} for both host and parasitoids species, indicating the ability to alter low temperature tolerance at short timescales, synonymous with rapid cold hardening (Kelty and Lee, 2001). This shows both the parasitoid and the host display short-term phenotypic plasticity of low temperature tolerance, and can acclimate in response to daily decreases in environmental temperatures (Kelty and Lee, 2001; Terblanche et al., 2007). However, the magnitude of plasticity following slower ramping rates was again asymmetrical, appearing higher in the host (pest) relative to the parasitoid, thus conferring survival advantage to the insect pest as opposed to its antagonist parasitoid. Nevertheless, we generally recorded no significant differences in CTLs between larval and adult of *P. xylostella*. This result is out of tandem with findings by Liu et al. (2002) and Chang et al. (2012) who observed higher thermal tolerance in larvae than adults. Indeed, ontogenetic differences in thermal tolerance have been recorded for different insect species (Mutamiswa et al., 2017; Schreven et al., 2017).

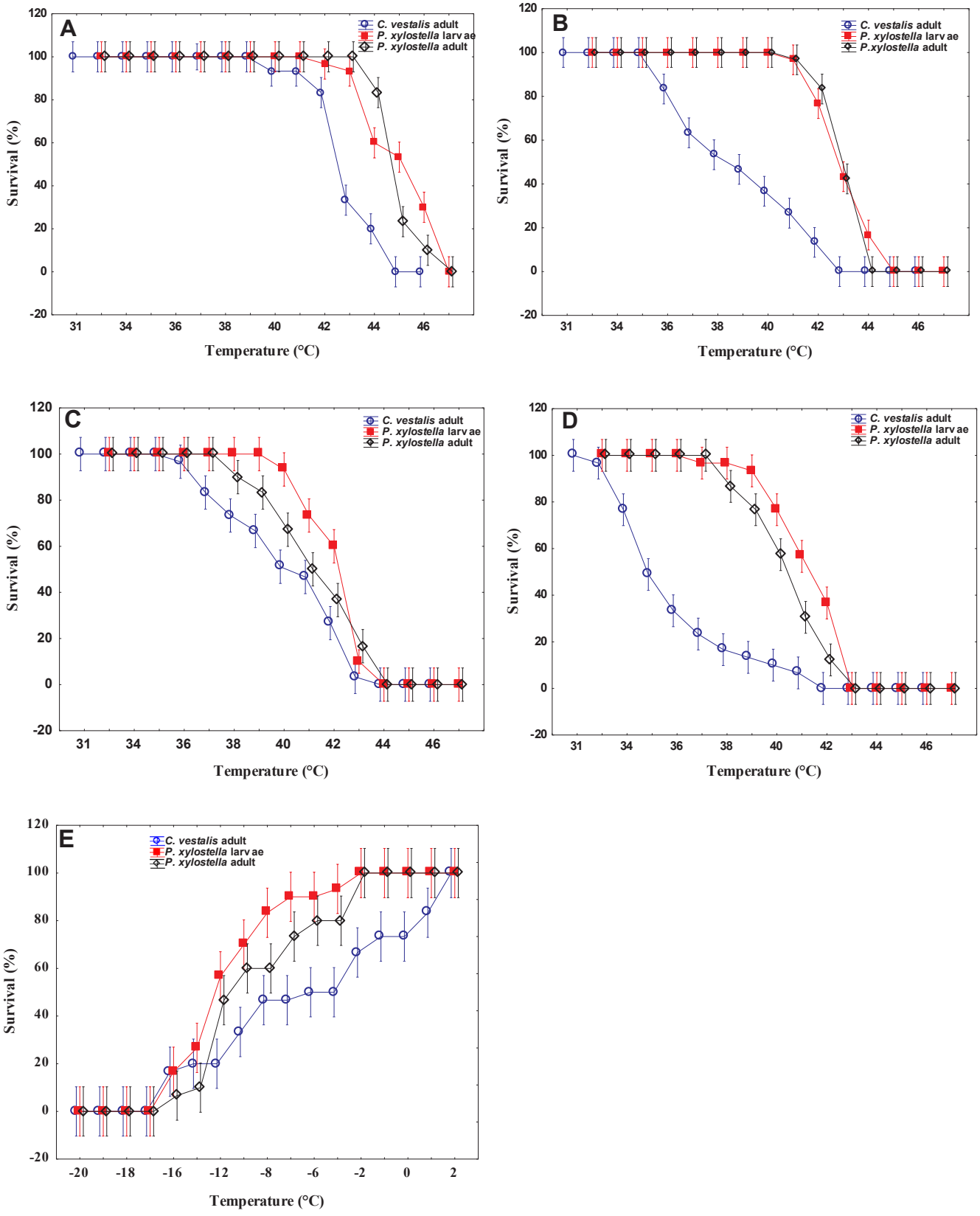

Fig. 3. Effect of treatment (*Cotesia vestalis* adults, *Plutella xylostella* larvae and adults) × time interaction effects on Upper Lethal Temperature (ULT) at durations (A) 0.5 h, (B) 1 h, (C) 2 h, (D) 4 h and (E) Lower Lethal Temperatures (LLT) for 2 h durations. Assays were done using plunge protocols (as in e.g. Chidawanyika and Terblanche, 2010). The area between the blue line and the red/black line denotes the degree of thermal divergence (overlaps) between the parasitoid (*C. vestalis)* and the host (*P. xylostella)*. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

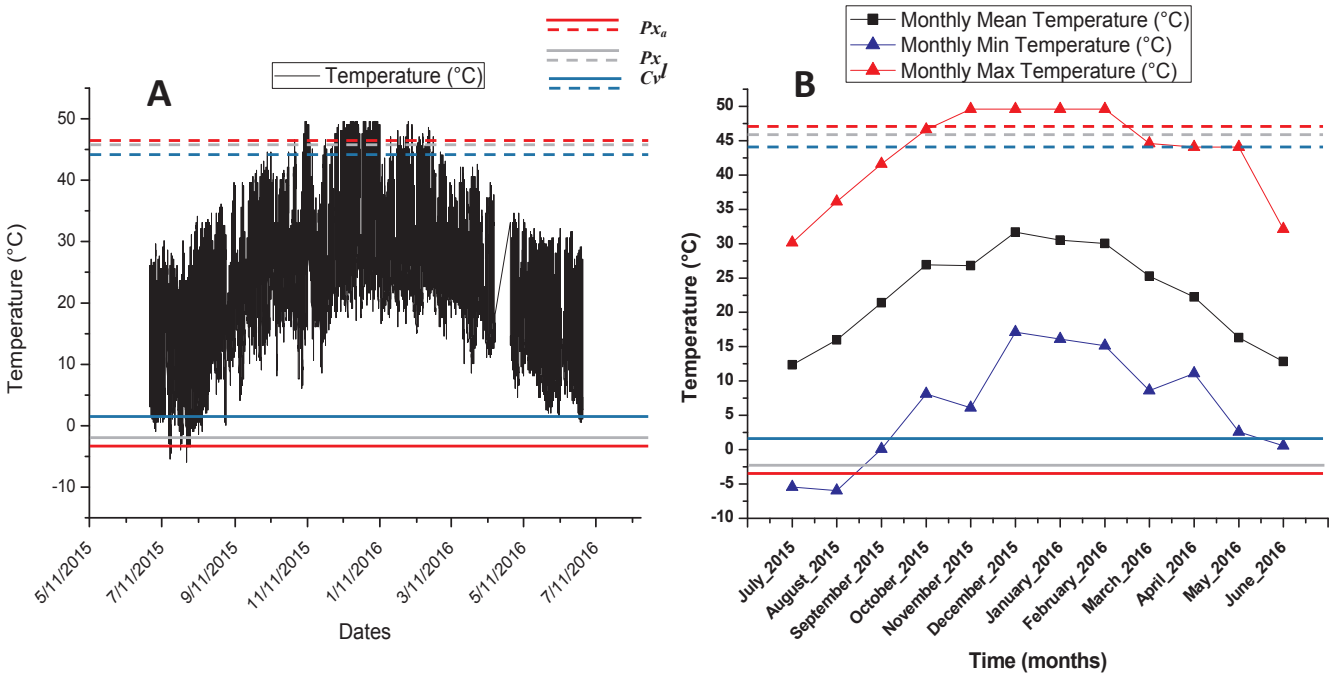

Fig. 4. Twelve months microclimatic shade temperature data recorded at Ambrose Phuthi Farm, Katleng District, Botswana (July 2015–June 2016). (A) shows hourly recorded temperature fluctuations while, (B) monthly mean, average monthly minimum and average monthly maximum temperatures. Dotted lines represent CT_{max} (blue = *C. vestalis (Cv)*, grey = *P. xylostella* larvae (*Px*$_l$) and red = *P. xylostella* adult (*Px*$_a$)), while solid lines represent CT_{min} (blue = *C. vestalis*, grey = *P. xylostella* larvae and red = *P. xylostella* adult). Where the graph line crosses the horizontal line(s), the recorded temperature is $\geq CT_{max}$ or $\leq CT_{min}$ for the given species. Temperature was recorded using Thermocron iButtons (Dallas Semiconductors, Model DS1920) (0.5 °C accuracy; 1 h sampling frequency). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

The reason for similar CTLs for both larva and adults in our study is thus unknown. In a related study, Zhang et al. (2015b) observed differences in heat tolerance between the early and the late larval instars of *P. xylostella*, citing thermal exposure history as the reason; e.g. eggs and first instar larvae are mainly found on the cooler underside of leaves. Similarly, lack of differences in our CTLs between 4th instar larvae and adults could be attributable to their thermal exposure history where both do not specifically prefer shaded areas due to continuous feeding (4th instar larvae), flying, feeding and mating (adult).

The HKDT and the CCRT are respective measures of heat and cold shock tolerance which are ecologically important traits of thermal adaptation (Ragland and Kingsolver, 2008) in a host-parasitoid system. The traits determine trophic level fitness especially under increased incidence of heat waves and cold snaps associated with global change (IPPC, 2014). They are an ecologically reliable measure of phenotypic resistance to thermal spectrum extremes especially in cosmopolitan trophic systems like that of *P. xylostella* and *C. vestalis* and are heritable putative targets of thermal selection for individual insect populations in nature (Ragland and Kingsolver, 2008; Bertoli et al., 2010). Our results showed lower HKDT for *C. vestalis* than *P. xylostella* larvae but not the adult and significantly longer recovery time for *C. vestalis* following chill coma. Heat stress disrupt metabolic and enzymatic activities, increase respiration to critical thresholds, disrupt the nervous system functions, affects cell membrane and nucleic acid function in insects leading to knockdown or death (Neven, 2000). Cold shock usually results in freezing of cellular and hemocoel fluids resulting in desiccation, ice induced damage on cellular membranes and tissues and enzymatic inactivity (Chen et al., 1987). In chill-comma recovery, high levels of internal body water content extends time required for desiccation (dehydration to critical levels that induce mortality) and the failure to efficiently re-establish haemolymph ion homeostasis (MacMillan and Sinclair, 2011). Differential HKDT and CCRT recorded between the host and the parasitoid may either be due to species differences in body water content as *C. vestalis* has less body water than both life stages of *P. xylostella* or due to heat and cold tolerance genes in *P. xylostella* (Shi et al., 2013; Zhang et al. (2015a,b)see discussions in ;). *Plutella xylostella* has one central quantitative trait loci mechanistic of both HKDT and CCRT.

Mechanisms underlying *P. xylostella* and *C. vestalis* differences in CCRT reported here remain unclear. Nevertheless, MacMillan and Sinclair (2011) revealed recovery from chill-comma is associated with the ability to re-establish ion homeostasis. The parasitoid *C. vestalis* had significantly longer CCRT than its host indicating a higher cold tolerance for the host insect pest. This may suggest species-related differences in maintenance and re-establishment of ion homeostasis (Mutamiswa et al., 2017), essential for chill-coma recovery. Similar studies have indicated interspecific variation in maintenance of water and ion homeostasis accounts for differences in *Drosophila* species cold tolerance in the laboratory (MacMillan et al., 2015) and field setting (MacMillan et al., 2016) (see discussions in Mutamiswa et al., 2017). This, therefore, shows that the ability to maintain ion homeostasis may be significant for insects in the wild to cope with the diurnal temperature fluctuations involving chilling.

Our SCP results showed significantly higher (more positive) supercooling temperatures for *C. vestalis* adult than *P. xylostella* adult indicating more significant cold hardiness for the host relative to the parasitoids. Our results are consistent with reports by Sinclair et al. (2009) who reported no effects of body size on SCPs in *Chymomyza amoena*. *Plutella xylostella* adult showed highly depressed SCP than its larvae, consistent with reports on a closely related taxa, the Indian meal moth *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae) (Kaneko, 1995; Carrillo et al., 2005). This may be caused by ontogenetic differences in the ability to accumulate cryoprotective substances, e.g. polyols, proteins and sugars responsible for enhancing SCPs. Indeed, *P. xylostella* diet is predominantly sugar based, and this may likely explain depressed SCPs (Sinclair et al., 2009). Conversely, gut contents in the solid feeding larvae (Carrillo et al., 2005; Carrillo and Cannon, 2005) and the likely presence of ice-nucleating active bacteria present in *P. xylostella* larval alimentary canal (Kaneko, 1995), may have acted as nucleating agents, thus increasing the chances of ice formation at

relatively higher temperatures than in adults. Microclimate data recorded over 12 months showed that temperatures eliciting supercooling are not experienced in nature. Furthermore, LLT results showed mortality temperatures for both host and parasitoids are above supercooling temperatures, symbolising they may all be chill-susceptible. Thus, supercooling ability may not be that ecologically relevant in the current context, nevertheless, SCP results recorded here still confer a fitness advantage to the host relative to the parasitoid.

Lethal temperature assays are ecologically significant as they determine limits of activity, severity of tissue injury and the time required for recovery or repair of tissues (Chown and Nicolson, 2004; Chidawanyika and Terblanche, 2010). Our results showed a general reduction in survival with increased temperature stress magnitude and duration of exposure to both sub- and supra-optimal temperatures for both the parasitoid and the host. At all temperature × time interactions, *P. xylostella* survival was higher than that of *C. vestalis*, supporting existing reports (e.g. Dosdall et al., 2012; Schreven et al., 2017). Our results showed temperatures above 39 °C (at 30 mins duration) sufficed to cause significant mortality on *C. vestalis* relative to *P. xylostella*. The latter showed signs of significant mortality at 43 °C for the same duration. Our *P. xylostella* ULT_0 results agree with Nguyen et al. (2014) who reported ULT_0 of 42.6 °C. Nevertheless, to our knowledge, this is the first report, to date documenting ULT and LLT on *C. vestalis*. However, studies investigating effects of temperature on *C. vestalis* activity and development reported no activity or development at temperatures > 35 °C (Htwe et al., 2008) and > 38.2 °C (Bahar et al., 2013), consistent with the current results. This implies that in nature, persistently high average temperatures and heat waves may result in more *C. vestalis* mortality than *P. xylostella*, and this may offset the abundance of the parasitoid relative to its host and thus impacting negatively on the parasitoid ecological services.

Field microclimate temperature data revealed that both LLTs and ULTs are frequently surpassed in nature (Chidawanyika and Terblanche, 2010), for sufficient durations to cause mortality and this appears more severely so for the parasitoid than for the herbivorous host. Thus under Africa's rapidly changing climates (Lobell et al., 2008; Steynor et al., 2012; Metzger et al., 2013), the frequency of occurrence of lethally stressful temperatures is likely to be higher for *C. vestalis*, compromising its fitness and biological control efficacy. Current results predict a fitness advantage for *P. xylostella* relative to *C. vestalis* under thermal stress, consistent with reports on other *P. xylostella* parasitoids e.g. *D. insulare* (Dosdall et al., 2012; Bahar et al., 2013) and *D. semiclausum* (Schreven et al., 2017). However, using simulation models, Ngowi et al. (2017) observed otherwise. Current results thus predict challenges for biological control of *P. xylostella* under rapidly changing climates and recommends use of evolutionary physiology to improve parasitoids' resilience (Thomas and Blanford, 2003; Hance et al., 2007; Chidawanyika et al., 2012; Sørensen et al., 2012; Chidawanyika et al., 2017; Machekano et al., 2017).

While our results provide significant ecological interpretations, there is still need to interpret these results in a broader ecological context that includes behavioural and physiological thermal responses of these species across a wide range of geographic locations (see recommendations by Andrew et al. (2013), the 'Moran effect' (see Liebhold et al., 2004) and buffering effect of microclimates across a range of landscapes (Pincebourde and Woods, 2012; Potter et al., 2013; Pincebourde et al., 2016). In addition the assumptions made in direct species comparison regarding evolution of thermal tolerance may not take into account several biotic and other abiotic factors, including behavioural stress adjustment (Nyamukondiwa et al., 2010). For example in a host-parasitoid system, larval parasitoid survival depends on host body temperature (Thomas and Blanford, 2003), and parasitized hosts differ in thermal responses from non-parasitised ones (Bahar et al., 2013), implying a complex network of unexplained eco-physiological responses. Thus host-parasitoid responses to temperatures are likely complex, as some hosts are more active with temperature increase (e.g. Dosdall et al., 2012; Bahar et al., 2013; Schreven et al., 2017) while some parasitoids are favoured by the same (Karban, 1998). Thus, effect of temperature on host-parasitoid systems is context-dependent and cannot be generalised across species or different trophic levels. Nevertheless current results provide critical insights on the thermal divergence on an economically important host-parasitoid system. The success of Integrated Pest Management of *P. xylostella* hinges on the effectiveness of future biological control which predominantly relies on parasitoids. The latter's field fitness and hence efficacy largely depends on its thermal tolerance profile. Therefore scope exists in the use of evolutionary physiology in pest management (see Hance et al., 2007; Chidawanyika et al., 2012; Sørensen et al., 2012; Machekano et al., 2017). Results support the potential for incorporating thermal conditioning in biocontrol programmes to improve temperature resilience of factory-reared *C. vestalis* parasitoids meant for mass release in biological pest management programs.

Declaration of interest

The authors declare they do not have any conflict of interests.

Submission declaration and verification

This work has not been previously published.

Authors' contributions

Conceptualization: CN, BMM.
Data curation: HM.
Formal analysis: CN, HM, BMM.
Funding acquisition: CN.
Investigation: HM, BMM, CN.
Methodology: HM, CN.
Project administration: CN.
Resources: CN.
Supervision: CN, BMM.
Validation: HM, BMM, CN.
Visualization: HM, BMM, CN.
Writing – original draft: HM, CN.
Writing, review & editing: HM, BMM, CN.

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